

CLAIMS

We claim:

1. A substantially purified RFX4_v3 polypeptide.
2. The polypeptide of claim 1, wherein the polypeptide comprises:
 - a) an amino acid sequence at least 70% identical to an amino acid sequence set forth as SEQ ID NO: 8;
 - b) a conservative variant of the amino acid sequence set forth as SEQ ID NO: 8; or
 - c) the amino acid sequence set forth as SEQ ID NO: 8, wherein the polypeptide has RFX4_v3 activity, and the N-terminus of the polypeptide is at least 90% identical to residues 1-14 of SEQ ID NO: 8.
3. The polypeptide of claim 2, wherein the polypeptide comprises an amino acid sequence set forth as SEQ ID NO: 6, or SEQ ID NO: 10.
4. The polypeptide of claim 2, wherein the polypeptide comprises an amino acid sequence set forth as SEQ ID NO: 8, or a sequence having at least 95% sequence identity to SEQ ID NO: 8.
5. An isolated nucleic acid molecule encoding the polypeptide of claim 2.
6. The nucleic acid of claim 5, wherein the nucleic acid molecule comprises:
 - a) a nucleic acid sequence at least 70% identical to the nucleic acid sequence set forth as SEQ ID NO: 37
7. The nucleic acid of claim 6, wherein the nucleic acid sequence is at least 90% identical to SEQ ID NO: 38 or SEQ ID NO: 39.
8. The nucleic acid of claim 6, wherein the nucleic acid sequence is at least 90% identical to SEQ ID NO: 37.
9. The nucleic acid sequence of claim 5, wherein the nucleic acid sequence is operably linked to a heterologous promoter.
10. The nucleic acid sequence of claim 5, wherein the heterologous promoter comprises SEQ ID NO: 11 or SEQ ID NO: 12.
11. A vector comprising the nucleic acid of claim 5.

12. A host cell transformed with the vector of claim 11.
13. The host cell of claim 12, wherein the host cell is a plant cell, an animal cell, or a
5 prokaryotic cell.
14. A composition comprising the polypeptide of claim 2.
15. An isolated nucleic acid molecule that hybridizes under conditions of low stringency
10 to a target nucleic acid molecule selected from the group consisting of nucleotides 1-42 of SEQ ID NO:
37, SEQ ID NO: 38, and SEQ ID NO: 39, wherein the isolated nucleic acid molecule is at least 15
nucleotides in length.
16. The isolated nucleic acid molecule of claim 15, that hybridizes under conditions of
15 high stringency to the target nucleic acid molecule.
17. The nucleic acid of claim 15, wherein the target nucleic acid molecule encodes a
RFX4_v3 polypeptide.
18. The nucleic acid of claim 12, wherein the RFX4_v3 polypeptide comprises SEQ ID
20 NO: 6, SEQ ID NO: 8, or SEQ ID NO: 10.
19. A vector comprising the nucleic acid of claim 15.
20. A host cell transformed with the vector of claim 19.
21. The host cell of claim 20, wherein the host cell is a plant cell, an animal cell, or a
prokaryotic cell.
22. The polypeptide of claim 2, wherein the RFX4_v3 activity comprises inhibiting the
30 phenotypic expression of congenital hydrocephalus.
23. The polypeptide of claim 2, wherein the activity is the ability to bind to RFX4_v3
specific antibodies.
24. The polypeptide of claim 2, wherein the polypeptide comprises the amino acid
35 residues set forth in SEQ ID NO: 33, SEQ ID NO: 34, or SEQ ID NO: 35.
25. A method for producing a variant of a RFX4_v3 polypeptide, wherein the method
40 comprises:

mutagenizing the wild-type nucleic acid sequence of SEQ ID NO: 37, SEQ ID NO: 38, or SEQ ID NO: 39; and
screening the variant for a RFX4_v3 activity.

5 26. A composition comprising a nucleic acid molecule that inhibits the binding of the first 42 nucleotides of SEQ ID NO: 37, SEQ ID NO: 38, or SEQ ID NO: 39 to its complementary sequence.

10 27. A polynucleotide sequence comprising at least fifteen nucleotides capable of hybridizing under stringent conditions to an isolated nucleotide sequence to nucleotides 1-42 of SEQ ID NO: 37.

 28. A method for detecting a nucleic acid molecule in a biological sample, wherein the nucleic acid molecule encodes a RFX4_v3 polypeptide, the method comprising:
15 hybridizing a polynucleotide to the nucleic acid molecule to produce a hybridization complex, wherein the polynucleotide hybridizes to nucleotides 1-42 of SEQ ID NO: 37, SEQ ID NO: 38, or SEQ ID NO: 39;
 detecting the hybridization complex, wherein the presence of the hybridization complex indicates the presence of a polynucleotide encoding RFX4_v3 in the biological sample.

20 29. The method of claim 28, wherein the polynucleotide hybridizes to SEQ ID NO: 37.

 30. The method of claim 30, further comprising amplifying the nucleic acid prior to hybridizing with the polynucleotide.

25 31. A method of identifying a subject at risk of developing RFX4_v3 linked hydrocephalus, comprising detecting in the subject an abnormality in a RFX4_v3 polypeptide or in a RFX4_v3 nucleotide sequence that alters expression of the RFX4_v3.

30 32. The method of claim 31, wherein detecting an abnormality comprises detecting a mutation in a nucleic acid sequence that encodes RFX4_v3, wherein the mutation is associated with RFX4_v3 linked hydrocephalus.

 33. The method of claim 31, wherein detecting an abnormality in the nucleic acid
35 comprises performing a hybridization analysis with a nucleic acid probe that detects the mutation in the RFX4_v3 nucleic acid sequence.

 34. The method of claim 31, wherein detecting an abnormality comprises identifying an individual carrying a mutated RFX4_v3 allele, wherein the method comprises:

providing a nucleic acid from a subject, wherein the nucleic acid comprises a RFX4_v3 allele;
and
detecting a mutation in the nucleic acid that results in phenotypic expression of congenital
hydrocephalus.

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35. The method of claim 34, wherein the mutation is in the RFX4_v3 allele.

36. The method of claim 31, wherein the method comprises detecting an abnormality in a
RFX4_v3 polypeptide.

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37. The method of claim 36, wherein the method comprises detecting an abnormality in
expression of the RFX4_v3 polypeptide.

38. The method of claim 37, wherein the abnormality in expression comprises detecting
a reduced expression of the RFX4_v3 polypeptide.

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39. The method of claim 36, wherein the method comprises providing a polypeptide
from a subject, and detecting a mutation in the polypeptide sequence, wherein the mutation results in
phenotypic expression of congenital hydrocephalus.

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40. The method of claim 31, comprising obtaining a biological sample from the subject,
and detecting in the biological sample the abnormality in the RFX4_v3 polypeptide or in the RFX4_v3
nucleotide sequence.

41. The method of claim 40, wherein the biological sample comprises blood, amniotic
fluid, plasma, or cerebral spinal fluid.

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42. The method of claim 40, wherein the method comprises:
providing a polypeptide from a subject, wherein the polypeptide comprises a gene product of a
RFX4_v1 gene; and
detecting a mutation in the polypeptide sequence,
wherein the mutation results in phenotypic expression of congenital hydrocephalus.

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43. The method of claim 42, wherein detecting the mutation in the polypeptide sequence
comprises detecting an abnormal protein or level or protein expression using RFX4_v1 specific
antibodies.

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44. A kit for determining if a subject is a carrier of a mutated RFX4_v3 gene, wherein
the kit comprises:

a reagent that specifically detects a mutation in a RFX4_v3 allele, and

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instructions for determining whether the subject is at increased risk of expressing congenital hydrocephalus if the reagent specifically detects the mutation.

45. The kit of claim 44, wherein the reagent comprises a nucleic acid probe that
5 specifically hybridizes under stringent conditions to a nucleic acid sequence of SEQ ID NO: 37, SEQ ID NO: 38 or SEQ ID NO: 39.

46. The kit of claim 44, wherein the reagent comprises an antibody that specifically binds
10 the protein expressed by the RFX4_v3 allele.

47. A method for generating antibodies specific for an RFX4_v3 polypeptide, wherein
the method comprises injecting an animal with an RFX4_v3 polypeptide or an immunogenic portion
thereof.

48. The method of claim 47, further comprising preparing a hybridoma that expresses the
15 monoclonal antibody.

49. An RFX4_v3 specific antibody for use as a detection or therapeutic agent.

50. A method for generating a non-human transgenic animal with a knockout for the
20 RFX4_v3 gene, wherein the method comprises disrupting an RFX4_v3 transcript, the disruption being
sufficient to produce hydrocephalus in the transgenic animal.

51. The method of claim 50, wherein the non-human transgenic animal is a mouse.
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52. The method of claim 50, wherein disrupting a RFX4_v3 transcript comprises:
deleting or substituting any portion of the RFX4_v3 transcript,
inserting an exogenous gene into the RFX4_v3 transcript, or
any combination thereof.
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53. The method of claim 50, wherein disrupting the RFX4_v3 transcript comprises
crossing one non-human transgenic animal with a second non-human transgenic animal.

54. A transgenic mouse whose somatic and germ cells comprise a disrupted endogenous
35 RFX4_v3 gene, the disruption being sufficient to produce an increased susceptibility to developing
congenital hydrocephalus.

55. The transgenic mouse of claim 54, wherein the disrupted gene is introduced into the
mouse of an ancestor of the mouse at an embryonic stage, wherein the mouse, if homozygous for the
40 disrupted gene, does not reproduce.

56. The transgenic mouse of claim 54, wherein the disruption is an insertion within the RFX4_v3 gene.

5 57. The composition of claim 54, wherein the disruption is a deletion or substitution within the RFX4_v3 gene.

58. A method for screening compounds for the ability to alter RFX4_v3 activity, wherein the method comprises:

- 10 a) providing:
- i) a first polypeptide sequence comprising at least a portion of RFX4_v3,
 - ii) a second polypeptide sequence comprising at least a portion of a protein known to interact with RFX4_v3, and
 - iii) one or more test compounds; and
- 15 b) combining in any order the first polypeptide sequence comprising at least a portion of RFX4_v3, the second polypeptide sequence comprising at least a portion of a protein known to interact with RFX4_v3, and one or more test compounds under conditions such that the first polypeptide sequence, the second polypeptide sequence, and the test compound interact; and
- 20 c) detecting the presence or absence of an interaction between the polypeptide sequence comprising at least a portion of RFX4_v3 and the polypeptide sequence comprising at least a portion of a protein known to interact with RFX4_v3.

59. A pharmaceutical composition for treating congenital hydrocephalus comprising:

- 25 a) a therapeutically effective amount of a RFX4_v3 nucleic acid, polypeptide, or a therapeutically effective variant or portion thereof; and
- b) a pharmaceutically acceptable carrier.

60. A pharmaceutical composition for preventing congenital hydrocephalus comprising:

- 30 a) a RFX4_v3 nucleic acid, polypeptide, a variant, or a portion thereof, and
- b) a pharmaceutically acceptable carrier.

61. A method of treating congenital hydrocephalus in a subject, comprising administering to the subject a therapeutically effective amount of an agent that increases presence of a RFX4_v3 polypeptide in the brain of the subject.

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62. The method of claim 61, wherein the method comprises administering exogenous RFX4_v3 polypeptide to the subject.

63. The method of claim 61, wherein the method comprises increasing expression of RFX4_v3 polypeptide in the subject.

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64. The method of claim 63, wherein the method comprises introducing into the subject a vector that expresses the RFX4_v3 polypeptide in the subject.